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An AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid) for micellar delivery of hydrophobic drugs

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Abstract

An AB block copolymer of oligo(methyl methacrylate) (oMMA) and poly(acrylic acid) (PAAc) has been synthesized. The block copolymer forms micelles in an aqueous medium, as confirmed by a fluorescence probe technique using pyrene. Doxorubicin hydrochloride was incorporated into the micelle and the release profile of doxorubicin hydrochloride was investigated. Slow and prolonged release of doxorubicin hydrochloride from the micelle was observed. The AB block copolymer micelle can be useful for prolonged mucosal drug delivery of hydrophobic drugs. © 1998 Elsevier Science B.V.

Keywords: Block copolymer; Micelle; Oligo(methyl methacrylate); Poly(acrylic acid)

1. Introduction

In the past two decades, many researchers have investigated synthetic polymers as drug delivery carriers, since they have compositional diversity and can be prepared in a variety of forms, and many have good biocompatibility [1]. We have studied a soluble carrier system that forms a physical gel network upon body contact; this carrier was prepared by grafting a thermally-sensitive polymer to the backbone of a bioadhesive polyelectrolyte, poly(acrylic acid) (PAAc) for controlled ocular delivery [2]. We have also reported on hydrophobically-modified PAAc hydrogels, prepared by grafting oligomers of methyl methacrylate (oMMA) to the backbone chains in a PAAc hydrogel, for prolonged delivery of hydrophobic drugs or cationic proteins [3]. We have extended that last study to a soluble PAAc graft copolymer system [4]. Oligomers of MMA and co-oligomers with hydroxyethyl methacrylate (HEMA) were grafted to a soluble PAAc backbone. Loading and release of hydrophobic drugs or a cationic protein were investigated with that formulation [4].

In this present study, we have synthesized an AB block copolymer of oMMA and PAAc using a novel synthetic method. PAAc [5] and oMMA [6] are known to be biocompatible materials. The schematic structure of the AB block copolymer is shown in Fig. 1. This AB block copolymer should form a micellar structure in which a hydrophobic core (oMMA) is surrounded by the bioadhesive polyelectrolyte (PAAc) outer shell [7,8]. As the micelles are formed by intermolecular association of single polymer units, they should eventually be dissociable into single polymer chains, for excretion from the body.

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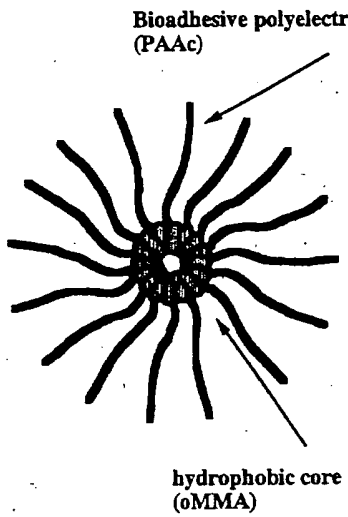

Fig. 1. The schematic structure of an amphiphilic AB block copolymer in an aqueous medium.

The advantage of polymer micelles over conventional surfactant micelles is the good thermodynamic stability in physiological solutions of the former, as indicated by their low critical micellar concentration (CMC) [7]. Similar block copolymers with glassy hydrophobic segments, such as polystyrene, form stable micelles, and the release rate of the single polymer chains from the micelle is expected to be slowed by the glassy structure of the core [7]. The micelle should be useful as a drug carrier for hydrophobic drugs [8–23] especially if the drug acts as a plastisizer in the micellar core. Furthermore, our technique permits us to vary the composition of the hydrophobic block, which controls the Tg of the core before drug loading. The drug itself will affect the Tg of the core, and that is another variable that should be optimized in any delivery system utilizing such micelles.

Kataoka and his colleagues have extensively investigated polymeric micelles as carriers of anticancer drugs [8–20]. They synthesized AB block copolymers of poly(ethylene oxide) and L-benzyl aspartate that formed micelles with diameters in a range of several tens of nanometers, mimicking viruses, in order to prevent reticuloendothelial systems (RES) recognition when administered intravenously to the body [7]. They reported enhanced tumor accumulation, prolonged circulation times [16] and reduced toxicity [12] of the AB block copolymer micelle when the aspartate block was conjugated with pendant adriamycin molecules instead of benzyl groups. In these studies, although adriamycin was covalently coupled within the micelle core in most cases, it could also be physically entrapped within the hydrophobic core of the micelle [18]. Akashi and his colleagues have studied graft copolymer nanoparticles having hydrophobic backbones and hydrophilic branches [24]. Akiyoshi, Sunamoto and colleagues reported cholesterol-conjugated polysaccharide aggregates for use as a drug carrier [25–28]. When the drug was covalently coupled within the hydrophobic core of the micelle, it was difficult to control the cleavage rate of the drug linkage.

In this present study, doxorubicin hydrochloride was physically entrapped in the hydrophobic oMMA core of our micelle. From a practical point of view, a micellar formulation has many advantages; for example, it can be administered not only via oral, topical and percutaneous routes but also via the parenteral route. Moreover, it has potential for site-specific drug delivery by conjugating targeting ligands onto the PAAc blocks. Furthermore, it may be bioadhesive due to the PAAc block which forms the outer shell of the micelle, and therefore especially suitable for topical or oral delivery. We present here our study of the synthesis, characterization and in vitro drug release behavior of the amphiphilic oMMA-b-PAAc block copolymer as a drug carrier.

2. Materials and methods

2.1. Materials

Methyl methacrylate (MMA), acrylic acid (AAc) and N,N-dimethylformamide (DMF) were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI, and were used after distillation under reduced pressure. 2,2'-Azobisisobutyronitrile (AIBN) and 2-amino ethanethiol hydrochloride (AET) were purchased from Aldrich Chemical Company, Inc., and used after recrystallization with methanol. Diethyl ether, tetrahydrofuran and pyrene were purchased from Aldrich Chemical Company, Inc., and used as received. Poly(methyl methacrylate) standards were purchased from American Polymer Standards Corpo-

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2.3. Preparation c
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2.2. Preparation of amino-terminated oligo(methyl methacrylate)

Amino-terminated oligo(methyl methacrylate) (oMMA) was synthesized by a free radical polymerization as reported previously [3]. Briefly, MMA was polymerized using AIBN and AET, as initiator and chain transfer agent, respectively in DMF solution. The molar ratio of MMA to AIBN to AET was 100 to 0.5 to 4. Prior to the reaction, the reaction mixture was subjected to repeated freeze thaw cycles in a sealed ampule. The reaction was carried out for 6 h at 60°C under vacuum. After the reaction, the oMMA was precipitated by addition of water and filtered. After washing with water, the precipitate was dried in vacuum.

The molecular weight of the amino-terminated oMMA was determined by gel permeation chromatography (GPC) using poly(methyl methacrylate) standards, as reported previously [3]. Briefly, GPC was carried out in tetrahydrofuran using a Waters 501 type pump, Millipore Corporation, Milford, MA, at a flow-rate of 0.7 ml/min at room temperature with a Waters Ultrastyragel 10^4 Å column (f7.8x300 mm), a Waters Ultrastyragel 10^3 Å column (f7.8x300 mm) and a Waters Ultrastyragel 500 Å column (f7.8x300 mm) (Millipore Corporation) connected in series. The polymers were detected by index of refraction with a Waters 410 Differential Refractometer, Millipore Corporation. The sample solution of 50ml was injected to the GPC system using a Waters U-6K Universal Injector, Millipore Corporation. The molecular weight of the amino-terminated oMMA was 4,300.

2.3. Preparation of an ab block copolymer of omma and paac

An AB block copolymer of oMMA and PAAc was synthesized by polymerizing AAc using AIBN and sulfhydryl-terminated oMMA as initiator and chain transfer reagent, respectively. The schematic reaction of the AB block copolymer is shown in Fig. 2. The amino-terminated oMMA of 2.15 g (0.5 mmole) was reacted with 0.404 g (1.0 mmole) of DSP in 20 ml of DMF solution and two molecules of the oMMA were connected by a disulfide bond. The reaction mixture was stirred for 24 h at room temperature. The dimer was reduced by adding 0.403 g (3.0 mmole) of DTT to the reaction mixture and the mixture was reacted for 24 h at room temperature to obtain the sulfhydryl-terminated oMMA. The sulfhydryl-terminated oMMA was precipitated by addition of water and filtered. After washing with water, the precipitate was dried in vacuum. Then 3.6 g (50 mmole) of AAc was polymerized using 0.041 g (0.25 mmole) of AIBN and 0.17 g (0.04 mmole) of the sulfhydryl-terminated oMMA as initiator and chain transfer reagent, respectively in 70 ml of a mixture of ethanol and water (80/20, w/w). The reaction was carried out at 50°C for 24 h. After the reaction, the AB block copolymer was precipitated by addition of diethyl ether and filtered. The precipitate was washed with diethyl ether and dried in vacuum.

2.4. Characterization of micelle formation using a fluorescence probe technique

The formation of micelles was confirmed by a fluorescence probe technique using pyrene [29,30]. The AB block copolymer and pyrene were suspended in distilled water, and fluorescence spectra and excitation spectra were measured using a fluorimeter (LS-5B, Perkin-Elmer, Norwalk, CT) for concentrations of the AB block copolymer varying from 0.0005 to 5 mg/ml. The fluorescence spectrum of pyrene at a fixed excitation wavelength (l_{ex}) of 339 nm, and the excitation spectrum at a fixed emission wavelength (l_{em}) of 390 nm were measured with constant pyrene concentration of $6.0x10^{-7}$ M [29].

2.5. Drug loading of the ab block copolymer micelle

Doxorubicin hydrochloride, an anti-cancer drug, was used as a model drug as it can be entrapped into

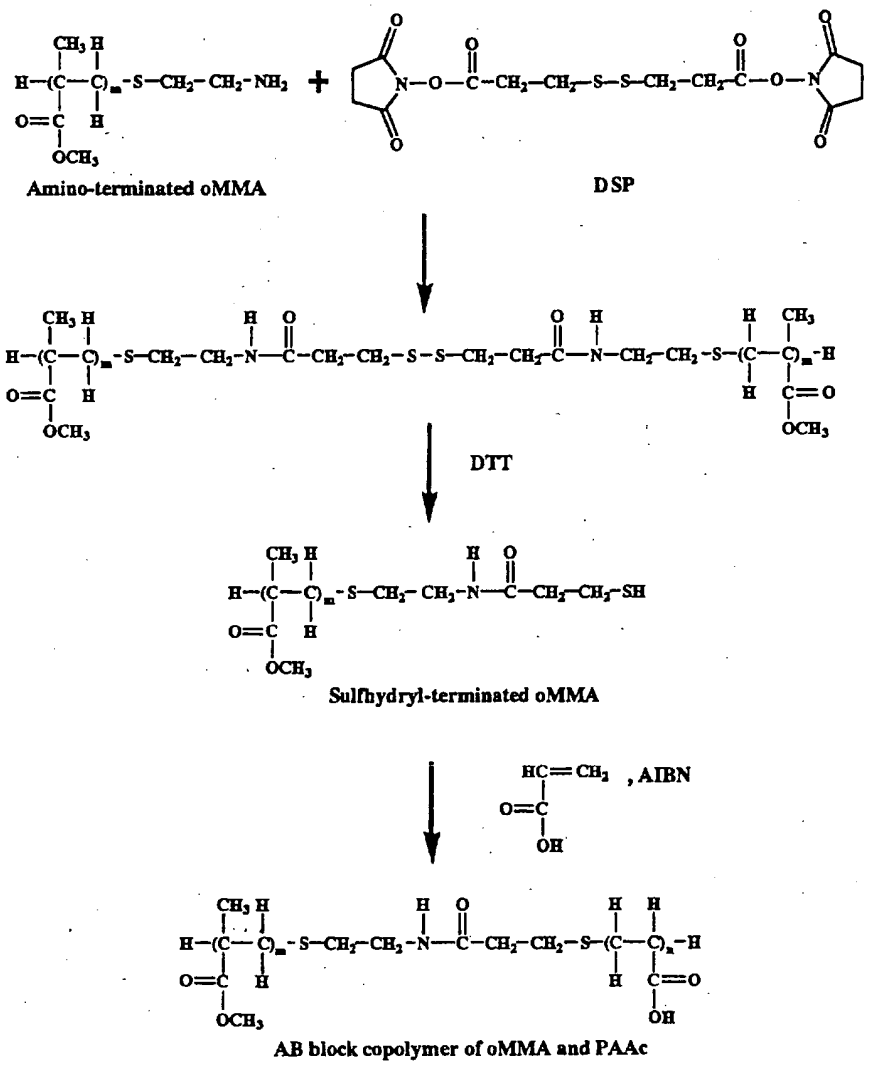

Fig. 2. Synthesis of an amphiphilic AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid).

the hydrophobic core of amphiphilic AB block copolymer micelle [18]. The doxorubicin hydrochloride loaded micelle was prepared by a simple dialysis technique [18]. The AB block copolymer of 0.02 g was dissolved in 4 ml of DMF together with 0.02 g of doxorubicin hydrochloride. The solution was put into a dialysis tube (molecular weight cut off: 3,500, Spectrum Medical Industries, Inc., Laguna Hills, CA) and subjected to dialysis against 1000 ml of distilled water over 24 h, while protecting the system from light to avoid decomposition of the doxorubicin. As DMF diffused out from the dialysis tube and water diffused in, doxorubicin hydrochloride was entrapped physically in the hydrophobic core of the AB block copolymer micelle, which formed as the degree of dilution with water increased and the block copolymers phase separated. The amount of doxorubicin hydrochloride was 2 mg/2 mg AB block copolymer/ml, which was determined after dilution by ethanol by measuring the absorbance at 485 nm

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2.6. Characterization of the micelle by gpc

The doxorubicin hydrochloride loaded micelle was characterized by GPC. GPC was carried out using a Waters Model 501 pump at a flow-rate of 0.7 ml/min at room temperature with a Waters Ultrahydrogel 250 column (f7.8x300 mm) and a Waters Ultrahydrogel 500 column (f7.8x300 mm), Millipore Corporation, connected in series, in 50mM phosphate buffered saline, pH 7.4 (PBS). The polymers were detected simultaneously with a Waters 410 Differential Refractometer, Millipore Corporation, and by absorbance at 485 nm with a Waters 484 tunable absorbance detector, Millipore Corporation. The sample solution of 50ml was injected to the GPC system using a Waters U-6K Universal Injector, Millipore Corporaion.

2.7. Doxorubicin hydrochloride release from the micelle

The doxorubicin hydrochloride release profile from the micelle was evaluated in PBS. The drug-loaded micelle suspension was put into a 1.5 ml polypropylene tube (Eppendorf, Hamburg, Germany). Then the tube was capped with a dialysis membrane (molecular weight cut off: 1000, Spectrum Medical Industries, Inc.) and put into a vial containing 10 ml of PBS and shaken by an Orbit Shaker (model 3540, Lab-Line Instruments, Inc., Melrose Park, IL) at a shaking speed of 100 rpm, at room temperature. The system was protected from light. Aliquots of 0.5 ml were withdrawn from the solution periodically. The volume of solution in the vials was held constant by adding 0.5 ml of the fresh PBS after each sampling. As a control, we used the identical concentration of doxorubicin hydrochloride in an aqueous solution in a 1.5 ml polypropylene tube capped with the dialysis tube. The amount of doxorubicin hydrochloride released from the tube was measured using the spectrophotometer at 485 nm. The fractional release of the drug was calculated as a function of time. All data points are averages of three determinations.

3. Results and discussion

3.1. Verification of micelle formation using a fluorescence probe technique

An AB block copolymer of oMMA and PAAc was synthesized by polymerizing AAc using AIBN and sulfhydryl-terminated oMMA as initiator and chain transfer reagent, respectively. The formation of micelles from the AB block copolymer was verified by a fluorescence probe technique using pyrene [29]. The fluorescence spectra of pyrene in the presence of the AB block copolymer at a fixed l_{ex} of 339 nm are shown in Fig. 3. The higher the AB block copolymer concentration, the higher the fluorescence intensity, which indicates micelle formation [29].

The excitation spectra of pyrene in the presence of the AB block copolymer at a fixed l_{em} of 390 nm are shown in Fig. 4. The higher the AB block copolymer concentration, the higher the fluorescence, especially at 339 nm, due to the fact that pyrene in water has a very small absorption at 339 nm, which increases when it is transferred into a hydrophobic environment. This effect also supports the proposed micelle formation [29]. Fig. 5 shows the effect of the AB block copolymer concentration on the intensity ratio of 339 nm/334 nm on the excitation spectrum. Although the intensity ratio of 339 nm/334 nm as a function of logarithm of the AB block copolymer concentration was constant below 10^{-1} mg/ml of the AB block copolymer, the intensity increased dramatically above that concentration, due to formation of micelles and the transfer of pyrene into the hydrophobic domain of the micelle. This concentration can be defined as the critical micellar concentration (CMC) [29]. Although the CMC of the AB block copolymer obtained in this study is higher than that of the poly(ethylene oxide)-poly(b-benzyl L-aspartate) copolymer micelle (CMC=5.0-10.0 mg/l) [14], it is much lower than that typical of poloxamers (CMC=1–24 g/l) [30], indicating that this micelle is relatively stable. From these results, we concluded that our AB block copolymer forms micelles in aqueous media. (A parallel conclusion was that we had indeed formed a block copolymer of oMMA and PAAc, since a physical mixture of the two would separate in water, and could not reproduce the fluorescence data with pyrene).

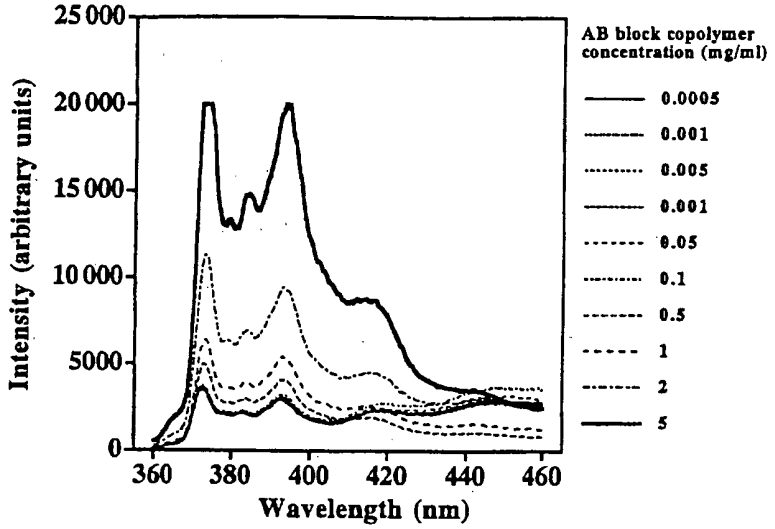

Fig. 3. Fluorescenece spectra of pyrene in the presence of an amphiphilic AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid) at a fixed excitation wavelength (l_{ex}) of 339 nm. The concentration of pyrene was 6.0×10^{-7} M and the AB block copolymer concentration was varied from 0.0005 to 5 mg/ml.

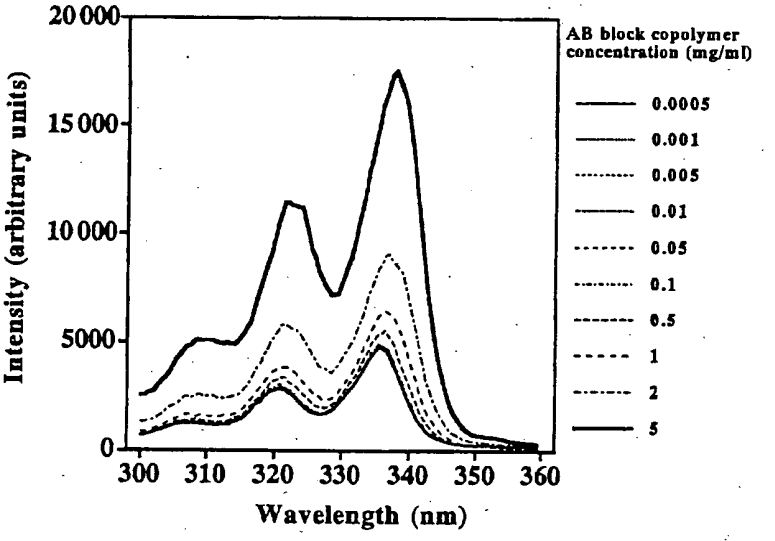

Fig. 4. Excitation spectra of pyrene in the presence of an amphiphilic AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid) at a fixed emission wavelength (l_{em}) of 390 nm. The concentration of pyrene was 6.0×10^{-7} M and the AB block copolymer concentration was varied from 0.0005 to 5 mg/ml.

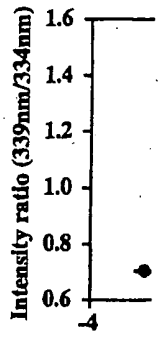

Fig. 5. The intensity spectra as a functio concentration.

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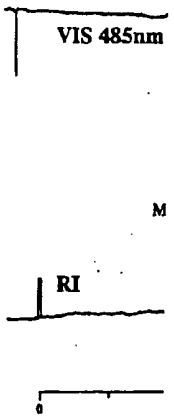

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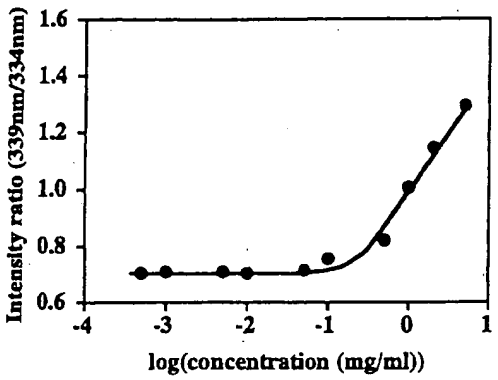

Fig. 5. The intensity ratio of 339 nm/334 nm in the excitation spectra as a function of logarithm of the AB block copolymer concentration.

3.2. Characterization of the micelle by gpc

The drug-loaded micelle was characterized by GPC. A typical GPC elution spectrum of the doxorubicin hydrochloride-loaded AB block copolymer micelle is shown in Fig. 6. The peak in index of refraction at MW of 2.3×10^6 probably represents the entrapped doxorubicin hydrochloride in the AB block copolymer micelle. It appears that most of the drug is entrapped in the micelle. The peak in index of refraction at MW of 4.1×10^4, which did not accompany the absorption at 485 nm is probably single polymer units unassociated with drug.

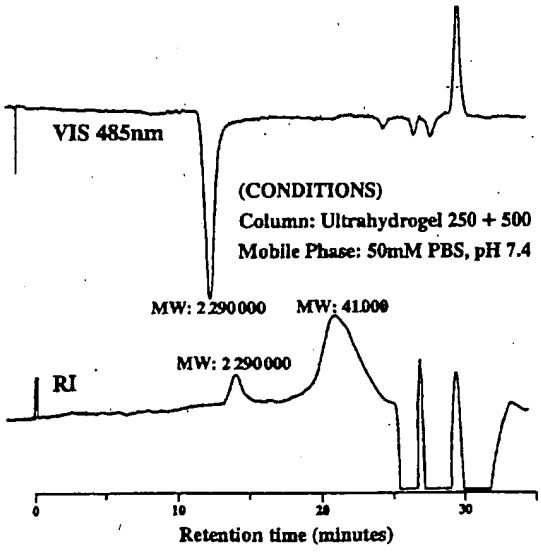

Fig. 6. A GPC spectrum of elution of a doxorubicin hydrochloride loaded AB block copolymer micelle.

3.3. Release of doxorubicin hydrochloride from the micelle

The release of doxorubicin hydrochloride from the micelle in PBS is slower than from a simple doxorubicin hydrochloride aqueous solution (which mimics use of a simple eye drop or nasal spray). (Fig. 7). The slow release of the drug from the control solution may be due to permeation barriers across the dialysis membrane. In addition to the same permeation barriers, the slowing release of the drug with time from the micellar solution may be due to the increase of the Tg of the core oMMA-drug mixture above 37°C as drug is released. It would be interesting to vary the composition of the core block so as to vary the glass transition temperature in the presence of the drug at 37°C. The Tg of the oMMA core before drug loading is expected to be similar to that of PMMA, that is, ca. 100°C.

An advantage of block polymer micelles over conventional surfactant micelles is the greater stability of the former in physiological solutions. AB block copolymers with glassy hydrophobic segments, such as PMMA or polystyrene, form especially stable micelles, and the release rate of the single AB polymer chains from the micelle is expected to be very slow due to the glassy phase in the core.

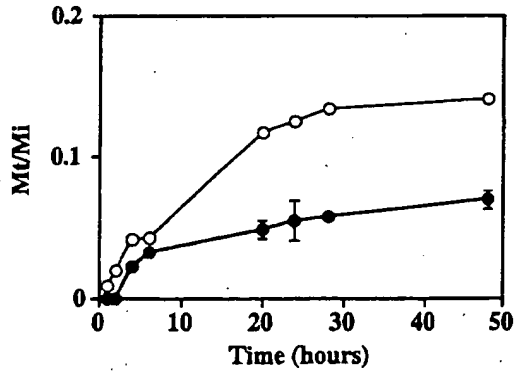

Fig. 7. Release of doxorubicin hydrochloride from an AB block copolymer micelle (●) and an aqueous solution (○) in phosphate buffered saline, pH 7.4 (PBS) at room temperature.

Another advantage of such a block copolymer micelle containing an outer shell of PAAc blocks is the potential for bioadhesion to mucosal surfaces. Thus, drug-loaded micellar solutions could be sprayed onto the eye, nose, mouth or throat, vagina or skin where the micelles would be expected to adhere and slowly release the drug.

It should also be noted that physical loading of hydrophobic drugs within the hydrophobic core of a micelle can be especially useful for drugs which have no suitable functional group, or where chemical conjugation of the drug to one block would inactivate the drug or release it at too slow a rate.

4. Conclusions

An amphiphilic AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid) has been synthesized using a novel synthetic method. The formulation of micelles in aqueous media was confirmed by a fluorescence probe technique using pyrene. Slow and prolonged release of physically entrapped doxorubicin hydrochloride from the micelle was observed. The Tg of the drug and core polymer will have a great influence on drug release rate from this micelle.

Acknowledgements

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